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Full Length Research Paper

Genetic diversity among endangered rare *Dalbergia cochinchinensis* (Fabaceae) genotypes in Vietnam revealed by random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers

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Dalbergia cochinchinensis **is a species of legume in the Fabaceae family. This species is popular and valuable in Vietnam and is currently listed on the Vietnam Red List and on the IUCN Red List as endangered. Genetic diversity of the 35 genotypes of *D. cochinchinensis* species were evaluated by using 52 markers random amplified polymorphic DNA and inter simple sequence repeats (27 RAPD and 25 ISSR). The RAPD primers produced 74 fragments in which 31 were polymorphic with an average of 1.148 polymorphic bands per primer. The number of amplified fragments varied from 1 (OPR15, OPB05, RA142, OPR08, UBC348, OPE14 and OPO04) to 8 (OPP19) and their sizes ranged from 250 to 1600 bp. Amplification of genomic DNA of the 35 genotypes using ISSR analysis yielded 94 fragments of which 64 were polymorphic with an average of 2.560 polymorphic bands per primer. The number of amplified fragments ranged from 1 (ISSR8, ISSR13 and ISSR18) to 6 (ISSR3, ISSR15 and ISSR61) and their size varied from 300 to 2000 bp. The estimated value of molecular diversity parameters within 35 genotypes such as the different number of alleles, effective number of alleles, Shannon's information index, intralocus gene diversity and Nei's gene diversity for RAPD were 1.149, 1.125, 0.109, 0.366 and 0.094, respectively; 1.284, 1.246, 0.206, 0.732 and 0.156, respectively in ISSR; 1.225, 1.193, 0.164, 0.542 and 0.124, respectively in RAPD + ISSR. The results indicate that both marker systems RAPD and ISSR, individually or combined can be effectively used in the determination of genetic diversity among *D. cochinchinensis*.**

Key words: *Dalbergia cochinchinensis*, endangered, genetic diversity, inter simple sequence repeats, random amplified polymorphic DNA.

INTRODUCTION

Dalbergia cochinchinensis is a leguminous tree species in the Fabaceae family, widely distributed in Cambodia, Laos, Thailand and southern Vietnam. It grows to 15 or even up to 30 m in height and it is a large evergreen tree of open semi-deciduous forests.

The wood of this tree has brown-red colour and is valuable for construction and ornamental work including wood decorations and furniture. In the market, the lumber is sold under the names Rose wood, Thailand Rosewood and Tracwood. In Vietnam, the huge demand for *D. cochinchinensis* has placed this species in danger of extinction at level EN A1a, c, d (Dang and Nguyen, 2007); it is forbidden to exploit, ship or store wood from this tree, according to decision 32/2006/ND-CP issued by the Vietnam government. It is considered to be a threatened species in Vietnam. In Thailand, the pressures on the species have also caused concern. Natural populations of *D. cochinchinensis* are disappearing and only limited numbers of individuals are found in the

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Table 1. Detail of *Dalbergia cochinchinensis* genotypes employed for the study of genetic diversity from different location of southern Vietnam.

Code number	Collected location of sample	Code number	Collected location of sample
Dc1	Yokdon National park	Dc19	Yokdon National park
Dc2	Yokdon National park	Dc20	Yokdon National park
Dc3	Yokdon National park	Dc21	Yokdon National park
Dc4	Yokdon National park	Dc22	Yokdon National park
Dc5	Yokdon National park	Dc23	Yokdon National park
Dc6	Yokdon National park	Dc24	Yokdon National park
Dc7	Yokdon National park	Dc25	Yokdon National park
Dc8	Yokdon National park	Dc26	Yokdon National park
Dc9	Yokdon National park	Dc27	Yokdon National park
Dc10	Yokdon National park	Dc28	Yokdon National park
Dc11	Yokdon National park	Dc29	Yokdon National park
Dc12	Yokdon National park	Dc30	Yokdon National park
Dc13	Yokdon National park	Dc31	Yokdon National park
Dc14	Yokdon National park	Dc32	Yokdon National park
Dc15	Yokdon National park	Dc33	KBang (Gia Lai)
Dc16	Yokdon National park	Dc34	KBang (Gia Lai)
Dc17	Yokdon National park	Dc35	KBang (Gia Lai)
Dc18	Yokdon National park		

remaining forest fragments of the southern part of Vietnam. Thus, in order to effectively plan gene conservation strategies, it is essential to have an understanding of the patterns of genetic variation in this population to protect it from further genetic corrosion. Over the years, the genetic diversity of forest trees has been detected and assessed extensively using both morphological and molecular methods. Several molecular marker techniques are now used in diversity studies in which the most commonly used systems are restriction fragment length polymorphism (RFLP) (Soller and Bechmann, 1983), random amplified polymorphic DNA (RAPD) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), inter-simple sequence repeats (ISSRs) (Zeitkiewicz et al., 1994) and microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994).

RAPD and ISSR markers are two widely applicable techniques to identify relationships at the species and cultivar levels (Arif et al., 2009; Gupta et al., 2008; Martins et al., 2003; Raina at al., 2001). RAPDs is an inexpensive, quick and simple technique (Williams et al., 1990). ISSRs was proposed for fingerprinting by Zeitkiewicz et al. (1994) and commonly used in population genetics, taxonomy and phylogeny of many plant species (Wolf and Randle, 2001). ISSR primers can also confirm specific amplified DNA polymorphic fragments within the variety (Li and Ge, 2001). Studies on biodiversity of valuable wood trees in *Dalbergia*, using RADP, ISSR, cpSSR and AFLP markers have also been implemented in several countries; for instance, Arif et al. (2009) comparative analysis of ISSR and RAPD markers for study of genetic diversity in Shisham (*Dalbergia sissoo*); Amri et al. (2009) evaluation of genetic diversity in *Dalbergia melanoxylon* populations using RAPD markers. Similarly, French, Indian and Brazilian researchers have applied RAPD and SSR markers, and specific genetic sequences to investigate genetic relationships among species and populations of *Dalbergia* (Juschum et al., 2007; Olivarimbola et al., 2004; Rout et al., 2003; Subhash and Manojkumar, 2004). In this study, we report the evaluation of genetic diversity among endangered rare *D. cochinchinensis* (Fabaceae) genotypes in Vietnam revealed by RAPD and ISSR markers.This information will aid in the long-term objective of efficient identification, conservation, exploitation and recreational use of this species.

MATERIALS AND METHODS

Thirty-two genotypes (Dc1-Dc32) collected from Yokdon National Park belong to the Dak Lak province the largest of Vietnam's nature preserves and is one of the seven internationally important Centers of Plant Diversity in Vietnam. This park encompasses over 1,000 km^2 and extends from eastern Cambodia into northern Dak Lak and southern Gia Lai provinces in Vietnam. The topography of most of this park is flat, with an elevation of approximately 200 m. Three genotypes (Dc33, Dc34 and Dc35) were collected from evergreen forest in KBang district. It is the largest forest of Gia Lai province with an area of 124,000 ha and a diversity of flora and fauna. The distance from the Gia Lai province to Dak Lak province is about 300 km^2 (Table 1, Figure 1). Thirty-five *D. cochinchinensis* geno-

Figure 1. Location of the *Dalbergia cochinchinensis* population in the Yokdon National Park (Dak Lak) and KBang (Gia Lai).

types were used for this study (named Dc1 to Dc35). These genotypes were classified into two different groups for analysis. Total genomic DNA was isolated from leaves and wood using the method described by Porebski et al. (1997).

The concentration of DNA was determined with a ultra violet (UV)-visible light spectrophotometer (UVS 2700, Labomed, USA), and the DNA samples were diluted to 20 ng μl^{-1} and used as templates for PCR amplification.

RAPD marker amplification

Arbitrary decamer primers used in this study were obtained from Alpha DNA, Canada (Table 2). PCR amplifications were performed in 25 µl volumes using a GeneAmp PCR System 9700 (Applied Biosystems USA). The reaction mixtures contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 10 to 20 ng genomic DNA, 10 pmol of primer, 2 to 4 mM $MgCl_2$, 300 to 400 µM of each dNTP and 0.8 to 1.2

Table 2. Genetic variation of 35 genotypes of *Dalbergia cochinchinensis* revealed by random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers.

S. No.	Primers code	Primer sequence	Amplified product range (bp)	*PIC	Poly. bands	Mono Bands	Total bands	Average Expected gene Diversity (*Hj*)
1	OPC19	GTTGCCAGCC	400-750	0.029	1	1	2	0.101
2	OPR15	GGACAACGAG	600	0.000	0	1	1	0.000
3	OPP08	ACATCGCCCA	400-1200	0.270	3	1	4	0.328
4	OPB05	GTGAGGCGTC	850	0.000	0	1	1	0.000
5	OPP19	GGGAAGGACA	250-1400	0.213	7	1	8	0.308
6	OPN05	ACTGAACGCC	300-800	0.000	0	6	6	0.000
7	OPC05	GATGACCGCC	400-1600	0.399	4	0	4	0.118
8	OPN16	AAGCGACCTG	900-1200	0.005	1	2	3	0.019
9	OPG13	CTCTCCGCCA	400-700	0.000	0	3	3	0.000
10	OPD13	GGGGTGACGA	400-1000	0.057	3	1	4	0.168
11	RA142	CCTTGACGCA	700	0.000	0	1	1	0.000
12	OPE20	AACGGTGACC	600-1100	0.132	1	1	2	0.248
13	OPB10	CTGCTGGGAC	300-500	0.000	0	2	2	0.000
14	OPR08	CCCGTTGCCT	500	0.000	0	1	1	0.000
15	OPD20	ACCCGGTCAC	300-800	0.157	2	1	3	0.321
16	UBC25	TGTAGCTGGG	300-800	0.000	0	3	3	0.000
17	OPH03	AGACGTCCAC	450-1400	0.210	2	1	3	0.294
18	OPD03	GTCGCCGTCA	700-1400	0.000	0	3	3	0.000
19	UBC348	CACGGCTGCG	400	0.000	0	1	1	0.000
20	OPA15	TTCCGAACCC	400-800	0.369	2	1	3	0.213
21	OPE14	TGCGGCTGAG	600	0.000	0	1	1	0.000
22	OPW13	TGCGCCCTTC	400-1000	0.312	4	2	6	0.220
23	OPH09	TGTAGCTGGG	300-800	0.000	0	2	2	0.000
24	OPO04	AAGTCCGCTC	750	0.000	0	1	1	0.000
25	OPP15	GGAAGCCAAC	550-700	0.233	1	1	2	0.204
26	OPV06	CTGCTGGGAC	750-950	0.000	0	2	2	0.000
27	OPN11	GAAACACCCC	400-1400	0.000	0	2	2	0.000
		Total		2.386	31	43	74	2.542
		Average		0.088	1.148	1.593	2.741	0.094
1	ISSR1	(CAG)5	400-1100	0.130	3	1	4	0.123
2	ISSR2	(CAA)5	600-1300	0.037	3	1	4	0.127

Table 2 Contd.

3	ISSR3	(GACA)4	400-1300	0.243	6	0	6	0.358
4	ISSR5	(CCG)6	450-1600	0.068	3	1	4	0.150
5	ISSR6	(CTC)6	450-1500	0.218	3	2	5	0.209
6	ISSR7	(GGC)6	400-1200	0.017	4	1	5	0.065
7	ISSR8	(GAA)6	500	0.000	0	1	1	0.000
8	ISSR10	(CTC)8	600-1200	0.041	1	2	3	0.118
9	ISSR11	(CCA)5	500-2000	0.225	3	1	4	0.268
10	ISSR12	(CCCT)4	500-800	0.000	0	2	2	0.000
11	ISSR13	(GT)8C	700	0.000	0	1	1	0.000
12	ISSR14	(CTCT)4GTC	400-1800	0.276	4	1	5	0.340
13	ISSR15	(CA)8A	400-1000	0.058	6	0	6	0.179
14	ISSR17	(CT)8T	400-600	0.000	0	2	2	0.000
15	ISSR18	(CT)8A	1000	0.000	0	1	1	0.000
16	ISSR46	(AG)8T	300-800	0.205	3	1	4	0.202
17	ISSR51	(GA)8A	400-800	0.044	3	1	4	0.149
18	ISSR55	(AC)8T	500-1000	0.047	1	3	4	0.113
19	ISSR56	(AC)8G	700-1200	0.106	3	1	4	0.173
20	ISSR61	(AC)8TG	400-1500	0.183	4	2	6	0.303
21	ISSR62	CTC(AG)7	300-600	0.250	3	1	4	0.189
22	ISSR63	CTC(GA)7	300-900	0.127	5	0	5	0.293
23	ISSR64	ACA(GT)7	300-950	0.000	0	2	2	0.000
24	ISSR67	(ATG)6	600-1200	0.117	4	1	5	0.281
25	ISSR69	(GGGTG)3	500-1300	0.141	2	2	4	0.250
		Total		2.533	64	31	95	3.89
		Average		0.101	2.560	1.240	3.800	0.156

units of Taq DNA polymerase (Amersham). The temperature profile consisted of an initial denaturation step at 94°C for 4 min, followed by 45 cycles: 92°C for 1 min, 35°C for 1 min and 72°C for 1 min. After the final cycle, samples were incubated for 10 min to ensure complete extension and then stored at 4°C. The PCR products were separated on 1.5% agarose gel in 0.5 × TBE buffer (Tris-borate-ethylenediaminetetraacetic acid). The size of amplified DNA fragments was estimated with 1 kb ladders (Fermentas, USA). The gels were visualized under UV using gel documentation (CSL-MiCRODOC, Cleaver, England).

ISSR marker amplification

ISSR primers for ISSR were obtained from Integrated DNA Technologies, USA (Table 2). The reaction mixtures contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 10 to 20 ng genomic DNA, 10 pmol of primer, 2 to 4 mM $MgCl_2$, 300 to 400 µM of each dNTP and 0.8 to 1.2 units of Taq DNA polymerase (Amersham). The temperature profile consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles: 94°C for 1 min, 38 to 55°C for 1 min and 72°C for 1 min. After the final cycle, samples were incubated for 10 min to ensure complete extension. The product was stored at 4°C. The PCR products were separated on 1.5% agarose gel in 0.5 × Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer. The size of amplified DNA fragments was estimated with 1 kb ladders (Fermentas, USA). The gels were visualized under UV using gel documentation (CSL-MiCRODOC, Cleaver, England).

Data analysis

DNA fingerprints were scored for the presence (1) or

Table 3. A comparative list of genetic diversity of 35 genotypes of *Dalbergia cochinchinensis* using RAPD, ISSR and RAPD + ISSR primers.

Primer	RAPD	ISSR	RAPD + ISSR
Numbers of primer used	27	25	52
Amplified product range (bp)	250-1600	300-2000	250-2000
Total number of polymorphic bands	31	64	95
Total number of monomorphic bands	43	31	74
Total number of bands	74	95	169
Percentage polymorphic (%)	41.89	67.37	56.21
Average number of bands/primer	2.741	3.800	3.250
Average number of polymorphic bands/primer	1.148	2.560	1.827

RAPD, Random amplified polymorphic DNA; ISSR, inter simple sequence repeat.

absence (0) of bands of various molecular weight sizes in the form of a binary matrix. The Simqual program was used to calculate Jaccard's coefficients (Jaccard, 1908); these were calculated as follows:

$S_{ij} = a/(a + b + c)$

Where, S*ij* is the coefficient of similarity between two individuals *i* and *j*; a is the number of fragments shared by samples; b represents amplified fragments in sample *i*; and c represents fragments in sample *j*.

GenAlEx software (Peakall and Smouse, 2006) was used to calculate a principal coordinates analysis (PCA) that plotted the relationship between distance matrix elements based on their first two principal coordinates. Regression between two matrices obtained with two marker types was estimated using Nei's genetic diversity index (Nei, 1973). This yielded the regression (r2) which is a measure of relatedness between two matrices. In this case, the matrix regression corresponds to two independently-derived dendrograms. The UPGMA-based dendrogram was constructed using NTSYS 2.0 software, version 2.0 (Rohlf, 1992). Win-Boot software (Yap and Nelson, 1996) was used to compute bootstrap-based P-values to assess the strength of evidence for clustering; this data was bootstrapped with 1,000 replications, a long Jaccard's coefficient. The polymorphism information content (PIC) of each locus was determined, as described by Weir (1990):

$PIC = 1 - \sum P_{ij}^2$

Where, *Pi* is the frequency of the *i*th allele in the genotypes.
Average expected gene diversity was calculated using the formula:

H*i* = (h*1* + h*2*)/total number of loci

Where, h*1* and h*2* (that is, $h_j = (1 - p^2 - q^2)$] represent intralocus gene diversity.

RESULTS

For RAPD analysis

Thirty-five genotypes as obtained from two different regions [Yokdon National park (Dak Lak) and KBang (Gia Lai)] of southern Vietnam were amplified using the 27 RAPD primers, the sequences of the primers and the number of bands generated by each primer is given in Table 2. PCR amplification of DNA yielded 74 DNA fragments that were scored in 35 genotypes. The number of amplified fragments varied from 1 (OPR15, OPB05, RA142, OPR08, UBC348, OPE14 and OPO04) to 8 (OPP19) and their sizes varied from 250 to 1,600 bp. Of the 74 amplified fragments, 31 were polymorphic with the average number of bands per primer and average polymorphic bands per primer to be 2.741 and 1.148, respectively (Table 3). The polymorphism percent of each primer ranged from 0 (OPR15, OPB05, OPN05, OPG13, RA142, OPB10, OPR08, UBC25, OPD03, UBC348, OPE14, OPH09, OPO04, OPV06 and OPN11) to 87.5 (OPP19), with an average percentage polymorphism of 41.89. The PIC value varied from 0 (OPR15, OPB05, OPN05, OPG13, RA142, OPB10, OPR08, UBC25, OPD03, UBC348, OPE14, OPH09, OPO04, OPV06 and OPN11) to 0.399 (OPC05), with an average of 0.088. Values of different number of alleles, effective number of alleles, Shannon's information index, intralocus gene diversity (H*j*) and Nei's gene diversity (H*i*) among 35 genotypes of *D. cochinchinensis* using 27 RAPD markers were 1.149, 1.125, 0.109, 0.366 and 0.094, respectively (Table 4). AMOVA helps in the partitioning of the overall RAPD variations among groups and populations within the group. Data reveal that greater proportion of total genetic variation existed among population within the groups (63%) rather than among groups (37%) (Table 5) indicating that there are more variations within the population. This is helpful in making strategy for evaluation and conservation. Figure 2 shows a representation of the extensive polymorphism observed among the *D. cochinchinensis* genotypes as revealed by RAPD primer OPP19 (Figure 2).

A dendrogram based on UPGMA analysis grouped the 35 genotypes into two main clusters (Figure 4A) with genetic similarity within clusters ranged from about 78 to 100% (Figure 4A). Clusters I comprise 3 genotypes (Dc28, Dc29 and Dc30) from Yokdon National Park. The cluster II has two sub-clusters (II.1 and II.2) comprising

Table 4. Genetic diversity parameters characterizing of *Dalbergia cochinchinensis* using RAPD, ISSR and RAPD + ISSR primers.

Primer	RAPD	ISSR	RAPD + ISSR
No. of different alleles	1.149	1.284	1.225
No. of effective alleles	1.125	1.246	1.193
Shannon's information index	0.109	0.206	0.164
Polymorphic information content (PIC)	0.088	0.101	0.095
Intralocus gene diversity (Hj)	0.366	0.732	0.542
Average gene diversity (Hi)	0.094	0.156	0.124

RAPD, Random amplified polymorphic DNA; ISSR, inter simple sequence repeat.

Table 5. Summary of AMOVA analysis based on RAPD, ISSR and RAPD + ISSR primers of *Dalbergia cochinchinensis*.

Source of variance	Primer	SS*	MS	Est. var.	Percentage
Among groups	RAPD	19.544	19.544	2.715	37
	ISSR	27.931	27.931	3.476	28
	RAPD+ISSR	47.475	47.475	6.191	31
Among population within groups	RAPD	153.542	4.653	4.653	63
	ISSR	292.469	8.863	8.863	72
	RAPD+ISSR	446.010	13.515	13.515	69

*SS: Sums of squares; MS: mean sums of squares; Est. Var: estimated variance. RAPD, Random amplified polymorphic DNA; ISSR, inter simple sequence repeat.

32 genotypes. Sub-cluster II.1 contains 26 genotypes (Dc3, Dc4, Dc5, Dc6, Dc7, Dc8, Dc9, Dc10, Dc11, Dc12, Dc13, Dc14, Dc15, Dc16, Dc17, Dc18, Dc19, Dc20, Dc21, Dc22, Dc23, Dc24, Dc25, Dc26, Dc27 and Dc31) collected from Yokdon National Park. The sub-cluster II.2 comprises 6 genotypes. Among them, 3 genotypes (Dc1, Dc2 and Dc32) collected from Yokdon National Park and three genotypes (Dc33, Dc34 and Dc35) collected from KBang (Gia Lai) are in a mini sub-cluster with genetic similarity ranging from about 98 to 100%; genotypes Dc33 and Dc34 appeared to be closest to each other, with a 1.00 similarity coefficient. The results of PCA analysis were comparable to the cluster analysis (Figure 5A). The first three most informative principal components explained 68.86% of the total variation.

For ISSR analysis

PCR amplification of DNA using 25 ISSR primers produced 95 DNA fragments that were in 35 genotypes. The number of fragments varied from 1 (ISSR8, ISSR13 and ISSR18) to 6 (ISSR3, ISSR15 and ISSR 61) and their sizes varied from 300 to 2000 bp. Of the 95 amplified fragments, 64 were polymorphic with the average number of bands per primer and average polymorphic bands per primer to be 3.800 and 2.560, respectively (Table 3). Percentage polymorphism ranged from 0 (ISSR8, ISSR12, ISSR13, ISSR17, ISSR18 and ISSR64) to 100 (ISSR3, ISSR15 and ISSR63), with an average percentage polymorphism of 67.37 and PIC values varied from 0 (ISSR8, ISSR12, ISSR13, ISSR17, ISSR18 and ISSR64) to 0.276 (ISSR14), with an average of 0.101. Values of different number of alleles, effective number of alleles, Shannon's information index, intralocus gene diversity (H*j*) and Nei's gene diversity (H*i*) among 35 genotypes of *D. cochinchinensis* species using 25 ISSR markers were 1.284, 1.246, 0.206, 0.732 and 0.156, respectively (Table 4). AMOVA helps in the partitioning of the overall RAPD variations among groups and among populations within the group. Data revealed that greater proportion of total genetic variation existed among population within the groups (72%) rather than among groups (28%) (Table 5). Figure 2 shows a representation of the extensive polymorphism observed among the *D. cochinchinensis* genotypes as revealed by RAPD primer ISSR6 (Figure 3).

A dendrogram based on UPGMA analysis grouped the 35 genotypes into two main clusters (Figure 4B) with genetic similarity within clusters ranged from about 67 to 97%. In Cluster I, only one genotypes Dc9 was collected from Yokdon National Park. The cluster II has two sub-clusters (II.1 and II.2) comprises 34 genotypes. Sub-cluster II.1 contains 5 genotypes (Dc7, Dc28, Dc29, Dc30 and Dc31) collected from Yokdon National Park. The sub-cluster II.2 comprises 29 genotypes. Among them, 26 genotypes (Dc1, Dc2, Dc3, Dc4, Dc5, Dc6, Dc8, Dc10, Dc11, Dc12, Dc13, Dc14, Dc15, Dc16, Dc17, Dc18

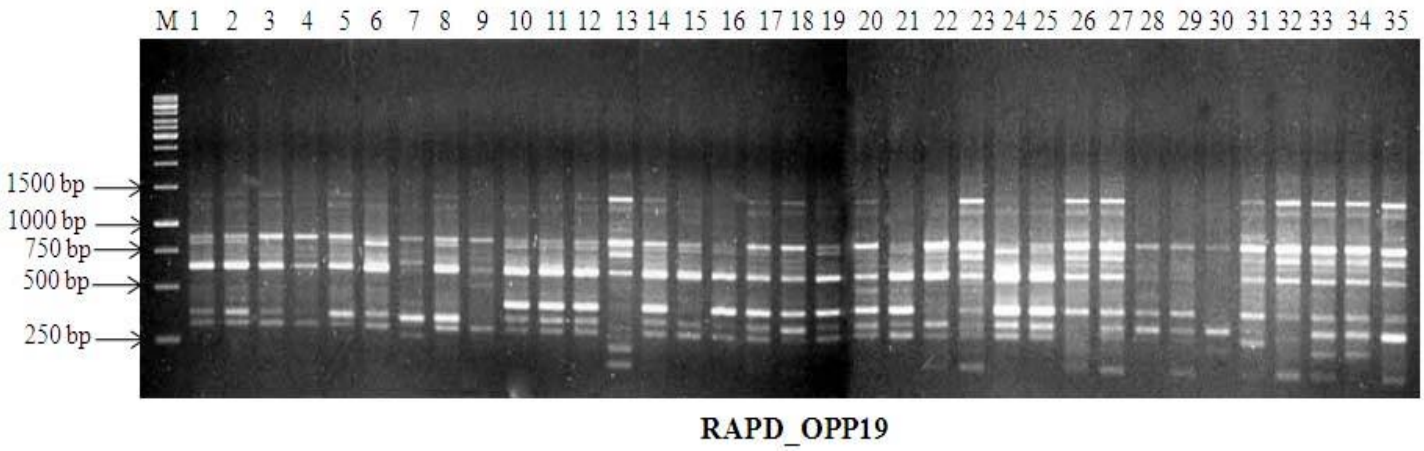

Figure 2. The RAPD profile of 35 *Dalbergia cochinchinensis* genotypes produced with primer OPP19 (Lane M is a 1 kb ladder and lanes 1 to 35 represent different *D. cochinchinensis* genotypes).

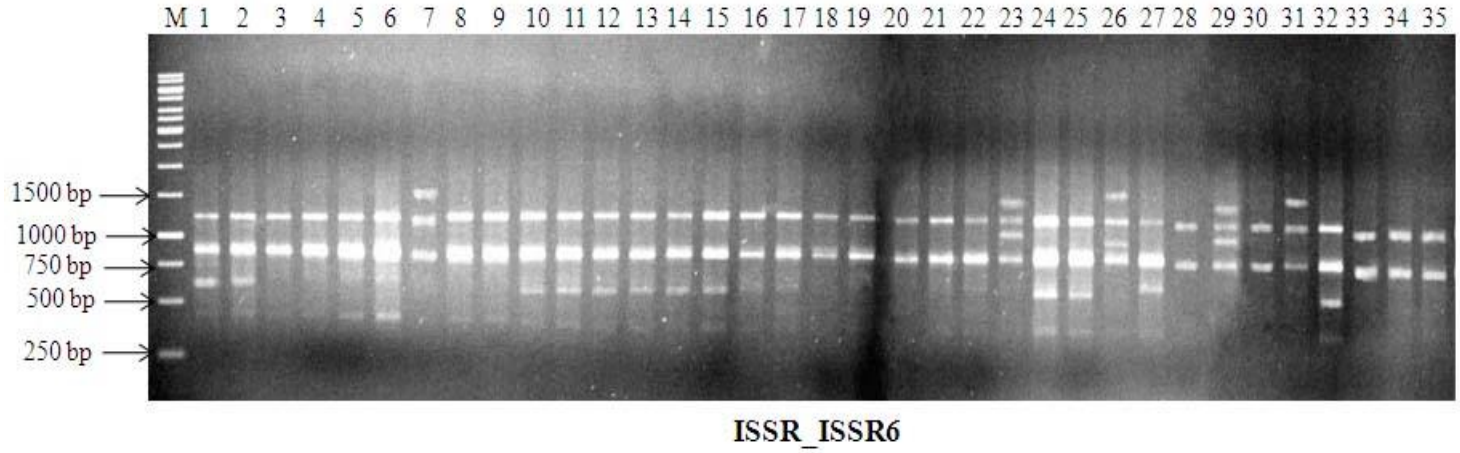

Figure 3. The ISSR profile of 35 *Dalbergia cochinchinensis* genotypes produced with primer ISSR6 (Lane M is 1 kb ladder and lanes 1 to 35 represent different *D. cochinchinensis* genotypes).

Dc19, Dc20, Dc21, Dc22, Dc23, Dc24, Dc25, Dc26, Dc27 and Dc32) were collected from Yokdon National Park, three genotypes (Dc33, Dc34 and Dc35) were collected from KBang (Gia Lai) into minisub-cluster with genetic similarity that ranged from about 94 to 97%. The results of PCA analysis were comparable to cluster analysis (Figure 5B). The first three most informative principal components explained 70.81% of the total variation.

Combined (ISSR and RAPD) data

The ISSR and RAPD data were combined for UPGMA cluster analysis. The dendrogram and cluster analysis gave a similar clustering pattern to that of RAPD + ISSR analysis, separately, with genetic similarity ranged from about 72 to 98% (Figure 4C). Thirty-five genotypes were grouped in two clusters in RAPD, ISSR and RAPD plus ISSR data. Interestingly, in all the three dendrograms, three genotypes (Dc33, Dc34 and Dc35) were collected from KBang (Gia Lai) into minisub-cluster. The results of PCA analysis were comparable to the cluster analysis (Figure 5C). The three most informative PCA components explained 71.08% of the total variation. Values of different number of alleles, effective number of alleles, Shannon's information index, intralocus gene diversity (H*j*) and Nei's gene diversity (H*i*) for all 35 genotypes using RAPD + ISSR markers were 1.225, 1.193, 0.164, 0.542 and 0.124, respectively (Table 4). AMOVA helps in partitioning of the overall RAPD + ISSR variations among groups and among populations within the group. Data reveal that greater proportion of total genetic variation

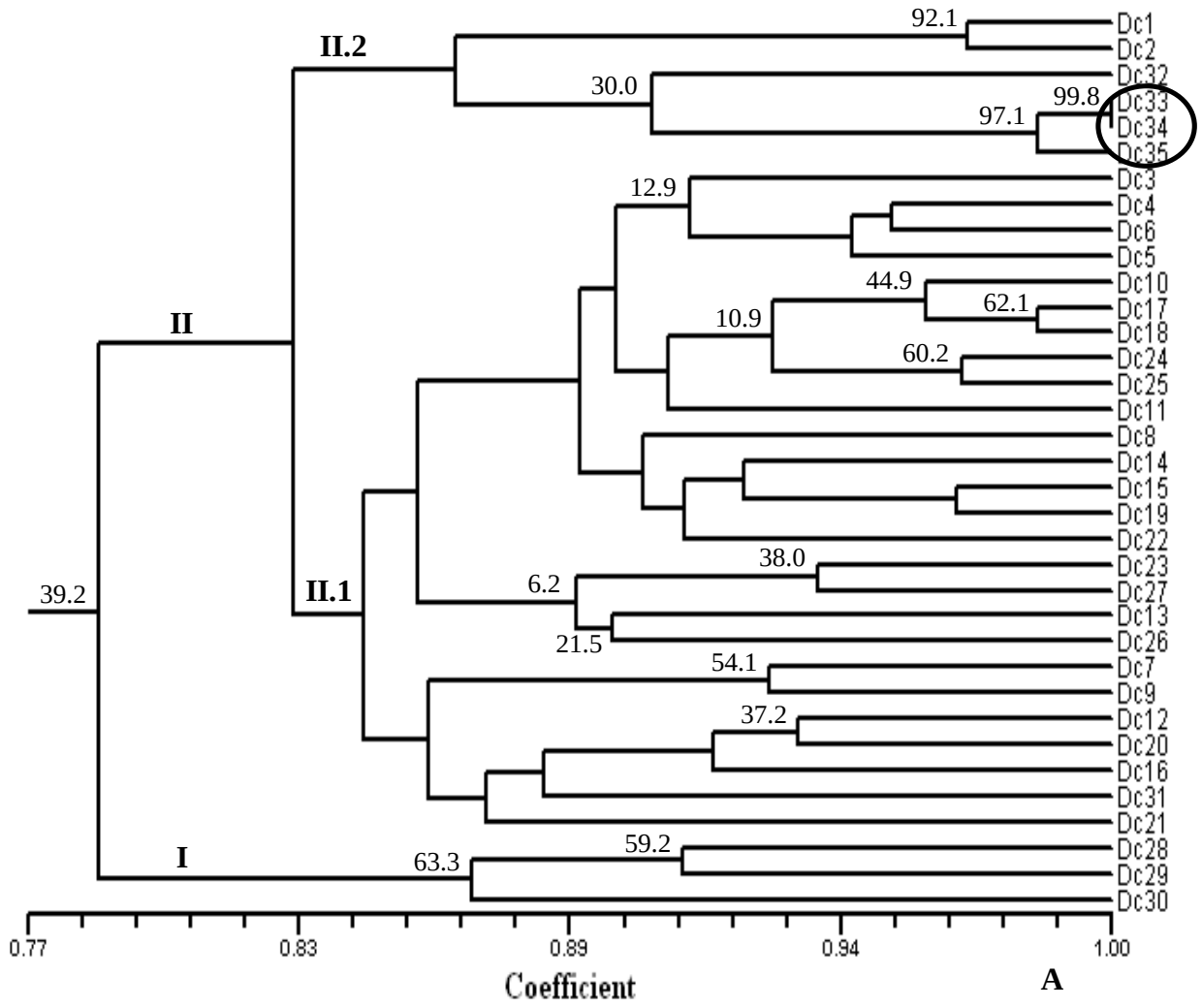

Figure 4. Dendrogram constructed using UPGMA based on Jaccard's coefficient. The numbers at the forks indicate the confidence limits for the grouping of those genotypes in a branch occurred based on 1,000 cycles in bootstrap analysis using the Winboot program. The scale bar indicates the similarity index; RAPD-data-based dendrogram (A); ISSR-data-based dendrogram (B); combined (ISSR and RAPD) data-based dendrogram (C).

existed among population within the groups (69%) rather than among groups (31%) (Table 5). Comparative analysis of RAPD with ISSR markers shows that ISSR markers were more efficient than RAPD assay in regards to polymorphism detection, as they detected 67.37% as compared to 41.89% for RAPD markers. Also, the average number of polymorphic bands per primer and total number of polymorphic bands are more for ISSR (2.560 and 64, respectively) than for RAPD (1.148 and 31, respectively).The different number of alleles, effective number of alleles, Shannon's information index, intralocus gene diversity (H*j*) and Nei's gene diversity (H*i*) estimates were more for ISSR (1.284, 1.246, 0.206, 0.732 and 0.156, respectively) than RAPD markers (1.149, 1.125, 0.109, 0.366 and 0.094, respectively).

DISCUSSION

In our studies, both RAPD and ISSR proved effective in genetic diversity research of *D. cochinchinensis* species. It is not surprising because these techniques have been used successfully in population genetic studies and in detecting genetic diversity in many species (Phong et al., 2011; Arif et al., 2009; Blair et al., 1999; Chen et al., 2006; Esselman at al., 1999; Gupta et al., 2008; Li and Ge, 2001; Moreno et al., 1998; Leian et al., 2005). Overall, ISSR markers exhibited the higher level of polymorphism or reproductive than RAPD as shown by our results (Phong et al., 2011; Esselman et al., 1999; Fang and Roose, 1997; Parsons et al., 1997; Qian et al., 2001). The polymorphism of the 35 genotypes of *D.*

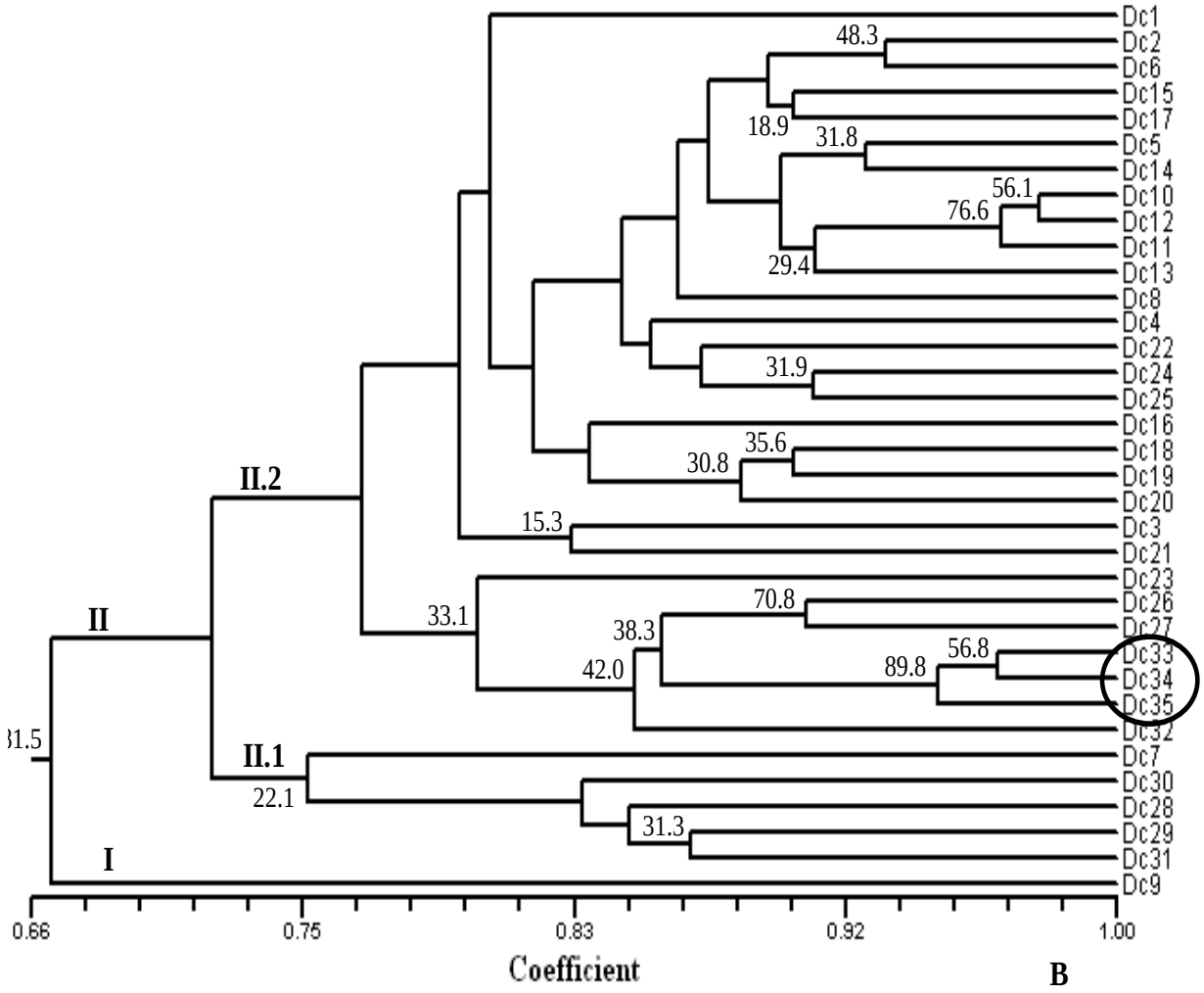

Figure 4. Contd.

cochinchinensis species was high. The percentage of ISSR polymorphic bands (67.37%) was higher than that of RAPD (41.89%). Also, the average number of bands and average number of polymorphic bands per primer are higher for ISSR (3.800 and 2.560, respectively) than for RAPD (2.741 and 1.148, respectively) (Table 3). Moreover, the total number of polymorphic bands (64) detected by 25 ISSR primers is much higher than that of the 27 RAPD primers (31).

The mean polymorphism information content (PIC) in ISSR analysis (0.101) was also higher than that in RAPD analysis (0.088). The result is similar in the report of Phong et al. (2011) who used both ISSR and RAPD markers in the assessment of genetic diversity in *Dalbergia oliveri* with PIC values (ISSR = 0.147 and RAPD = 0.116), the average expected gene diversity (ISSR = 0.169 and RAPD = 0.068) and the percentage of polymorphism bands (ISSR = 52.53% and RAPD = 25.57%). This finding is opposite to that reported by Arif et al. (2009) who worked with Shisham (*D. sisso*).

Some researchers have shown that ISSR markers are distributed throughout the genome and may be associated with functionally important loci (Esselman et al., 1999; Penner, 1996), while RAPD markers are located in non-coding regions and therefore are selectively neutral (Bachmann, 1997; Landergott et al., 2001). However, we cannot yet conclude that ISSR markers are functionally more important than RAPDs. This method is obviously advantageous in differentiating closely related cultivars and has been used for cultivar identification in numerous plant species including rice (Blair et al., 1999; Joshi et al., 2000), grapevine (Moreno et al., 1998), wheat (Nagaoka and Ogihara, 1997), apple (Goulaõ and Oliveira, 2001), mulberry (Zhao et al., 2006), barley (Hou et al., 2005) and strawberry (Arnau et al., 2003). RAPDs have been used for evaluating genetic

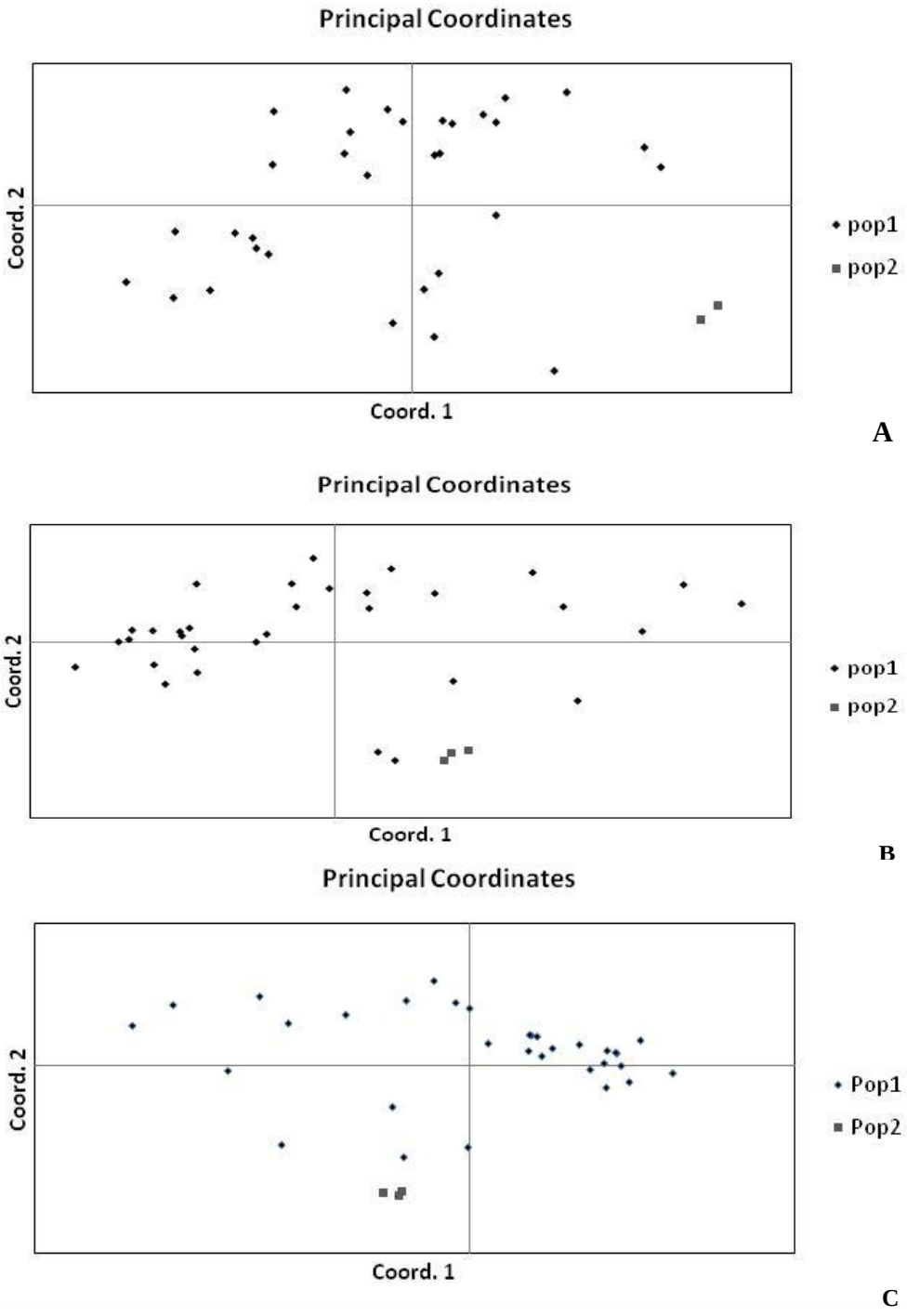

Figure 5. PCA across 35 *Dalbergia cochinchinensis* genotypes. RAPD-data-based PCA (A); ISSR-data-based PCA (B); combined (ISSR and RAPD) data-based PCA (C).

diversity within populations of *D. sisso*, *D. latifolia*, *D. paniculata*, *D. assamica* and *D. spinosa* (Rout et al., 2003). Andrianoelina et al. (2006) also quantified and analyzed the genetic variation of *Dalbergia monticola* using RAPDs. Compared to the other species in *Dalbergia* genus at the population level of genetic diversity using RAPD markers, Shannon's diversity index and Nei's genetic diversity were 0.239 and 0.358 for *D. sissoo* (Wang et al., 2011), 0.223 and 0.15 for *D. monticola* (Andrianoelina et al., 2006) and 0.2048 and 0.1353 for *D. odorifera* (Yang et al., 2007), while in our present study, they are 0.109 and 0.094 for *D.*

cochinchinensis (Table 4). However, the use of ISSR markers in the assessment the genetic diversity in *Dalbergia* was still limited. The recent report of Phong et al. (2011) have used 31 ISSR markers to analyse the genetic diversities of the three rare hardwood species of *Dalbergia* in Vietnam, Shannon's diversity index (I) and Nei's genetic diversity (H*i*) were 0.195 and 0.146 for *D. assamica*, 0.111 and 0.109 for *Dalbergia nigrescens*, 0.166 and 0.123 for *Dalbergia tonkinensis.* In this study, Shannon's diversity index and Nei's genetic diversity in ISSR analysis are 0.206 and 0.156, respectively for *D. cochinchinensis* (Table 4).

In our study, the relative genetic distances within species show the separation of samples from the geographical distances. Irrespective of the type of marker used (RAPD, ISSR or the combined RAPD + ISSR), three genotypes Dc33, Dc34 and Dc35 (collected from Gia Lai province) were grouped into a minor group, thirty-two genotypes Dc1 to Dc32 (collected from Yokdon National Park belong to the Dak Lak province) were grouped into the main group or subgroup (Figure 4A, B and C). It could be the reason for the observed genetic differentiation between the individuals of the *D. cochinchinensis* populations growing 300 km apart. This is one of the first studies on the DNA diversity of some species of *Dalbergia* genus in Vietnam. Our studies showed that apart from the high level of genetic diversity between species, a significant variation within a species was also found. This may be the result of a long-term of adaptation in diversity climatic and geographical conditions of Vietnam. Nevertheless, a further assignment should be established in more natural conditions to study the relationship between them and provide precise information for the protection of diversity.

Abbreviations

RAPD, Random amplified polymorphic DNA; **ISSR,** inter simple sequence repeats; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **SSR,** simple sequence repeat; **cpSSR,** chloroplast simple sequence repeat; **PCR,** polymerase chain reaction; **UPGMA,** unweighted pair group method with the arithmetic averaging algorithm; **PCA,** principal component analysis; **AMOVA,** analysis of molecular variance; **PIC,** polymorphic information content; **IUCN,** International Union for Conservation of Nature; **EN,** endangered.

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